AD-762 027

INHIBITION OF 3-O-METHYL GLUCOSE TRANS-PORT IN 'ASCARIS SUUM' MIDGUT BY 'ESCHERICHIA COLI' ENDOTOXIN

John H. Migliaccio

Oklahoma University Health Sciences Center

Prepared for:

Office of Naval Research

7 March 1973

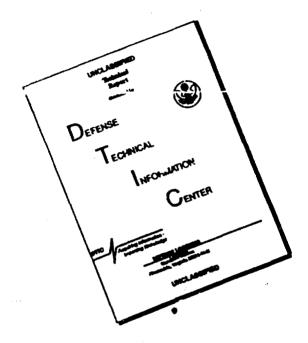
**DISTRIBUTED BY:** 



U. S. DEPARTMENT OF COMMERCE

5285 Port Royal Road, Springfield Va. 22151

## ISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

POL DATA P	t D	· · · · · · · · · · · · · · · · · · ·	
		toward competit to animals	
annetation must be e	28. REPORT 5' JURITY CLASSIFICATION		
MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.		UNCLASSIFIED	
		2b. GROUP	
		UNCLASSIFIED	
UNCLASSIF IED			
I IN <u>ASCARIS</u>	SUUM MILE	UT BY	
78. TOTAL NO. C	PAGES	76 NO OF HEFS	
89		5	
98. ORIGINATOR'S	REPORT NUM	BER(\$)	
70			
9b. OTHER REPORT NO(5) (Any other numbers that may be assigned this report)			
<u> </u>			
release and	sale; it	s distribution	
12. SPONSORING MILITARY ACTIVITY			
Office of Naval Research			
<del></del>			
ndotoxin conc is inhibited	entration is not k	. Despite the fact nown, the knowledge	
	PFICE INC.  IN ASCARIS  OF IN ASCARIS  OF IN ASCARIS  OF IN ASCARIS  70  OF IN ASCARIS  12. SPONSORING IN ASCARING IN AS	TINC.  UNCLASS  UNCLASS  UNCLASS  UNCLASS  UNCLASS  UNCLASS  UNCLASS  UNCLASS  UNCLASS  OF GROUP  UNCLASS  OF THE PAGES  OF THE	

DD FORM ., 1473

(PAGE 1)

ia

UNCLASSIFIED
Security Classification

A-31408

## INHIBITION OF 3-0-METHYL GLUCOSE TRANSPORT IN ASCARIS SUUM MIDGUT BY ESCHERICHIA COLI ENDOTOXIN

John H. Migliaccio

Technical Report No. 70 University of Oklahoma Health Sciences Center ONR Contract

March 7, 1973

Research sponsored by the Office of Naval Research Contract N00014-68-A-0496 Project NR 105-516

Reproduction in whole or in part is permitted for any purpose of the United States Government

This document has been approved for public release and sale; its distribution is unlimited

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.

Dogs were administered intravenous injections of endotoxin during septic shock studies conducted at the Veterans Administration Hospital in Oklahoma City, Oklahoma. Upon necropsy of animals that died of endotoxin shock, helminths found in the intestine were nonmotile and insensitive to mechanical stimulation. Following these observations two dogs, which were naturally infected with hookworms, Ancylostoma canum, and tapeworms, Taenia Faeniaeformis, were given endotoxin orally. Both animals passed parasites in their stools during the following 48 hours. These dogs were killed and at necropsy worms recovered from the gut were normotile.

extensively. However, the biochemical basis for endotoxic action has not been determined unequivocally (5). In view of this and the above observations in dogs, studies were carried out in an attempt to establish a specific mechanism of endotoxin action by investigating its effect on a helminth parasite in vitro. Ascaris sum was used as a model to study the effect of endotoxin on intestinal transport of 3-0-methyl glucose by parasitic worms. The intestine appears to be the principal route for nutrient uptake by nematodes (1-3). Beames determined the movement of several hexoses across the midgut of Ascaris and observed that movement of 3-0-methyl glucose from luminal to abluminal surface occurs against a concentration gradient when glucose is present in the incubation medium (1).

The thesis of this study was that the observed effects of endotoxin on intestinal helminths are due to altered nutritional status of the parasite.

The procedures followed in the experiments were patterned after those of Beames(1). Adult ascarids were collected at a local slaughter house and transported to the laboratory in saline (4), maintained between 32° and 39° C and were used within six hours. Individual worms were incised longitudinally with scissors. The reproductive system was pulled aside to expose the intestine which was then sectioned at the posterior portion. A Pasteur pipette was inserted into the lumen and secured with a silk ligature. Isolation of the middle third of the intestine was achieved by placing loops of silk thread around the intestine just beyond the tip of the Pasteur pipette and in the anterior region of the midgut. Material was introduced into the lumen of the intestine with a syringe fitted with a piece of polyethylene tubing which was inserted into the pipette. Fluid was forced into the intestine until it had moved past the most distant loop of thread. In this way the residual material in the intestine was flushed out of the preparation. The loops of thread were pulled tight and the resulting sac was cut free. The sac was blotted and placed in a vial containing 1.0 ml of glucose solution. The vial was stoppered with a 2-hole rubber stopper and flushed for 8-10 minutes with 95%  $\rm M_2$ :5%  $\rm CO_2$ , scaled and placed in a metabolic shaker for one hour at 37°C. Extra-luminal and intra-luminal solutions are shown in Table I. Endotoxin used in these experiments was a lipopolysaccharide of Eschericia coli (Control 262759, Difco Pharmaceutical Co., Detroit Michigan). Bile salts (glucochanodeoxycholic acid) were used to solubilize the endotoxin in Ascaris saline.

The bile salts solution was made in a 6mM concentration. Wasserman #1 paper was used and developed with butyl alcohol: pyridine: water (6:4:1/4) to detect the presence of any monosaccharides in the endotoxin and bile salts solutions. Both these solutions were found to be free of monosaccharides prior to mixing with a 3-0-methyl glucose for intraluminal injection.

The experimental results are shown in Table I. According to Beames (1), transfer of 3-0-methyl glucose was found to be 1.49 umoles/cm<sup>2</sup>/hr while the observed value in this experiment was 1.40 umoles/cm<sup>2</sup>/hr. Movement of 3-0-methyl glucose across the midgut of Ascaris was inhibited by endotoxin. Inhibition was dependent on endotoxin concentration. Despite the fact that the mechanism by which the transport is inhibited is not known, the knowledge that absorptive processes per se can be impaired may lead to a better understanding of the mechanism of endotoxin action.

## **ACKNOWLEDGEMENTS**

The author wishes to thank Dr. C. Beames, Department of Physiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, and Dr. G. A. Castro, Department of Physiology, University of Texas Medical Center at Houston, Houston, Texas, for their guidance and advice concerning the content of the study. A special thanks also to Dr. D. Holmes and Dr. J. Shmidl, Division of Comparative Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, for their encouragement and assistance.

## REFERENCES

- 1. Beames, 1971, J. Parasit. 57: 97-102.
- 2. Castro and Fairbairn, 1969, J. Parasit. 55: 13-16.
- 3. Fairbairn, 1957, Exp. Parasit. 6: 491-554.
- 4. Harpur, 1963, Can. J. Biochem. Physiol. 41: 1673-1689.
- 5. Nowthy, 1971, Naturarissenschafter 58: 397.

TABLE 1. Effect of Endotoxin on Transport of 3-0-Methyl Glucose Across Ascaris Suum Midgut

E	9	ស	m	
umoles 3-0-methyl glucose (±sx̄)	$1.40 \pm 0.32$	0.50 ± 0.05	0.29 ± 0.02	
Body Fluid	0.04M glucose	0.04M glucose	0.04M glucose	
Luminal Solution	0 µg endotoxin +)*	75 µg endotoxin (+)*	100 ng endotoxin (+)*	

\*0.04M C<sup>14</sup> 3-0-methyl glucose (+) 0.04M 3-0-methyl glucose (+) 6mM glycochenodeckycholic acid

the gut contained 0.04M  $\rm c^{14}$  3-0-methyl glucose (8985 dpm/umole). Gas phase 95% N2: 5%  $\infty$ 2, tamerature 37°C, time in metabolic shaker bath 60 min. (n) above indicates the number All solutions were prepared in Ascaris saline. The solution on the luminal side of of replicates.